



#18
dca
11/20/02

IN THE UNITED STATES PATENT & TRADEMARK OFFICE

Applicant(s): First et al.

Docket No.: 96429/9085

Serial No.: 09/463,276

Group Art Unit: 1632

Filing Date: May 12, 2000

Examiner: J. Woitach

Title: TRANS-SPECIES NUCLEAR TRANSFER

DECLARATION OF NEAL FIRST UNDER 37 CFR § 1.132

Assistant Commissioner for Patents
Washington, DC 20231

RECEIVED

Sir:

NOV 20 2002

I, Neal First, do hereby declare and state the following:

TECH CENTER 1600/2900

1. I am a Professor of Reproductive Biology in the Department of Animal Science, School of Agriculture at the University of Wisconsin-Madison, and co-inventor of all claims of the above-identified patent application. A copy of my curriculum vitae is attached.
2. I have reviewed the Final Office Action dated May 9, 2002, the Advisory Office Action dated September 23, 2002, and the references cited therein. The cited references include Gurdon (J. Cell Sci. Suppl. 4:287-318, 1986), Prather et al. (Biology of Reproduction 37:859-866, 1987), Campbell et al. (WO/97/07668), Telford et al. Molecular Reproduction and Development 26: 90-100, 1990), Dominko and First (Molecular Reproduction and Development 47:456-467, 1997), and Stice et al. (WO95/17500). Claims 12, 14, and 15 were rejected under 35 USC § 102(b) as being anticipated by Gurdon and claims 1-3 and 5-15 were rejected under 35 USC § 103(a) as being unpatentable over Prather et al., Gurdon, Campbell et al., Telford et al., Dominko et al., and Stice et al. For the reasons given below, I believe the present claimed invention is not taught or suggested by any one of the references or combination of references cited as bases for any of the rejections in the Final Office Action.

3. The invention as claimed is drawn to methods of producing embryos by interspecies nuclear transfer using a donor cell of a species other than bovine as the nuclear donor and an enucleated bovine oocyte as the recipient and to interspecies embryos that have undergone maternal to embryonic transition.

4. Gurdon is a review paper in which Gurdon cites his own studies using nuclear transfer in amphibians, as well studies of others using amphibian oocytes as the recipient and nuclear donors of other species. Gurdon teaches that development beyond the maternal to embryonic transition of amphibians reliably occurs if the nuclear transfer donor cell is from a pregastrula or pre-maternal to embryonic stage, but not if the donor cell is at the gastrula stage or has undergone maternal to embryonic transition. The maternal to embryonic transition of amphibians occurs at the twelfth cell cycle, whereas that of cattle and sheep occurs at the third cell cycle, that of primates and swine occurs at the second cell cycle, and that of rodents in the first cell cycle. Because of the differences in embryo development with respect to mechanisms, genomic imprinting, role of maternal cytoplasm, maternal control of positioning of cells in the embryo, and the like, the amphibian is not considered a good or true developmental model for mammals. One of skill in the art would not expect that the amphibian studies would predict the outcome of trans-species nuclear transfer into mammalian oocytes, particularly using bovine oocytes. Gurdon cites the work of Brun, who introduced a mammalian donor cell into an amphibian oocyte and produced a cleavage stage embryo, which did not pass the maternal to embryonic transition in development of either the donor or recipient species. Activation of an enucleated oocyte that had not received any donor nucleus would also result in a cleavage stage embryo.

5. I am an author on the Prather et al. paper cited against the claims. Prather et al. does not concern interspecies nuclear transfer, and teaches methods using early stage and pre-maternal to embryonic transition stage embryonic cells as nuclear donors. We used early embryonic cells, because we were unable to obtain advanced embryo development using advanced stage donor cells.

6. The Examiner cited Dominko et al. as demonstrating "increased efficiency in embryo development when the genetic material is transferred later than 8 hours of culturing". I am a co-author on the Dominko and First paper. The paper teaches a time for harvesting oocytes to be used in in vitro fertilization that results in recovery of oocytes of the highest developmental

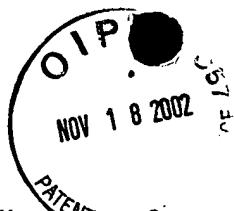
competence. This is a selection process that causes rejection of or discard of many fertilizable oocytes. To use the 16 hour oocyte in in vitro fertilization also requires a delay of 8 hours for maximum fertilization and development. Oocytes recovered at 24 hours can be fertilized immediately and require no 8 hour delay. The delay of 8 hours is not a pertinent delay in transferring a diploid nucleus. The oocytes of Dominko and First are not enucleated, and therefore contain a haploid nucleus and receive a second haploid nucleus from the sperm. Most importantly, the 8 hour delay is needed for a final maturation of the oocyte before it is fertilized. This is necessary only when the oocyte is recovered from maturation culture at the 16 hour stage, and coincides with the optimum time for fertilization of a 24 hour recovered oocyte.

7. As the Examiner acknowledged, Stice et al. does not teach interspecies nuclear transfer. Furthermore, Stice et al. does not use differentiated donor cells, as required by certain of the claims.

8. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements and the like so made are punishable by fine or imprisonment, or both, under Section 101 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Dated: 11-11-02

Neal L First
Dr. Neal First



Neal L. First

EDUCATION:

Michigan State College	1948-1952	B.S.	Animal Husbandry
Michigan State University	1954-1957	M.S.	Animal Science
Michigan State University	1957-1959	Ph.D.	Animal Science and Physiology of Reproduction

Ph.D. Thesis Title: Fertility of Frozen Ram Semen

PROFESSIONAL EXPERIENCE:

1956-60	Instructor, Michigan State University-East Lansing.
1960-64	Assistant Professor, Department of Meat and Animal Science, University of Wisconsin-Madison.
1964-1968	Associate Professor, Department of Meat and Animal Science, University of Wisconsin-Madison.
1968-present	Member, Endocrinology-Reproductive Physiology Program, University of Wisconsin.
1968-present	Professor, Department of Meat and Animal Science, University of Wisconsin-Madison.
1987-1990	Joint Appointment in Dept. of Obstetrics and Gynecology, Medical School.
1990-1992	Director USDA-CSRS-ARS National Animal Genome Mapping Program.

HONORS AND AWARDS:

Outstanding Teacher, University of Wisconsin, College of Agricultural and Life Sciences, 1968; Animal Physiology and Endocrinology Award, American Society of Animal Science, 1977; Outstanding Teacher, University of Wisconsin-Madison, 1978; Saddle and Sirloin Club Honorary Recognition Award, 1983; National Association of Animal Breeders National Research Award, 1986; Alexander von Humboldt Award, 1987; University of Wisconsin Distinguished Professor Chair, L.E. Casida Professor of Reproductive Biology and Biotechnology, 1989; Elected to National Academy of Science, 1989; SSR Research Award, 1991; ASAS Morrison Award, 1993; Wolf Prize, 1997.

NATIONAL COMMITTEES:

Acting Director, National Program in Mapping the Genome of Domestic Animals, 1990-1992; National Academy of Science, Institute of Laboratory Animal Research, 1991-1997; NAS Institute of Medicine Committee on Fetal Research and Application, 1992-1993; NAS Class Membership Committee, 1992-1993, 1999; National Advisory Board on Ethics in Reproduction, 1994-1998, NAS Commission on Life Sciences, 1997-2001; NCR, Standing Committee on Biotechnology, food and Fiber Production and the Environment, 2000- ; NRC, Chimpanzee Retirement Committee, 1997-1998; Chair, Coldsprings Harbor Banbury Workshop on Cloning Animals, 2000.

SELECTED PUBLICATIONS

Mitalipova, M., Beyhan, Z., First, N.L. 2001. Pluripotency of Bovine Embryonic Cell Line Derived from Precompacting Embryos. Submitted to Biology of Reproduction.

Memili, E., Segev, H., First, N.L. 2001. Control of Gene Expression at the Onset of Bovine Embryonic Development. Zygote. 9:123-133.

Kent, M.J. and First, N.L. 2000. Getting genes where we want them. Nature Biotechnology. 18:928-929.

Memili, E. and **First, N.L.** 2000. Zygotic and embryonic gene expression in cow: a review of timing and mechanisms of early gene expression as compared with other species. *Zygote*. 8:87-96.

Dominko, T., Mitalipova, M., Haley, B., Beyhan, Z., Memili, E., McKusick, B., & **N.L. First**. 1999. Bovine oocyte cytoplasm supports development of embryos produced by nuclear transfer of somatic cell nuclei from various mammalian species. *Biology of Reproduction*. 60:1496-1502.

First, N.L., Mitalipova, M., Kent-First, M.J. 1999. Reproductive Technologies and Transgenics. Book chapter in *The Genetics of Cattle*, CABI Publishing, NY, NY pp 411-437.

Beyhan Z, Johnson LA, **First NL**. 1999. Sexual dimorphism in IVM-IVF bovine embryos produced from X and Y chromosome bearing sperm sorted by high speed flow cytometry. *Theriogenology* 52 (1):35-48.

First, N.L. and Thomson, J. 1998. From cows stem therapies? *Nature Biotechnology* 16 (7): 620-621.

First, N.L. and Bremel, R. 1998. Transgenic Dairy Animals for Production of Novel Proteins in Milk. Book chapter in *Agricultural Biotechnologies, Principles and Practices*, Marcel Dekker, Publisher.

Fulka, J. Jr., **First, N.L.**, Loi, P., Moor, R.M. 1998. Cloning by somatic cell nuclear transfer. *Bioessays* 20:847-851.

Memili, E. and **First, N.L.** 1998. Developmental Changes in RNA Polymerase II in Bovine Oocytes, Early Embryos and Effect of α -amanitin on Embryo Development. *Molecular Reproduction and Development*. 51: 381-389.

Memili, E., Dominko, T. and **First, N.L.** 1998. Onset of Transcription in Bovine Oocytes and Preimplantation Embryos. *Molecular Reproduction and Development* 51:36-41.

Fulka, J. Jr., **First, NL**, Moor, R.M. 1998. Nuclear and cytoplasmic determinants involved in the regulation of mammalian oocyte maturation. *Molecular Human Reproduction* 4.1:41-49.

Dominko T and **First NL** 1997. Timing of meiotic progression in bovine oocytes and its effect on early embryo development. *Mol Reprod Dev* 47:1-12.

Dominko TD and **First NL** 1997. Relationship between the maturational state of oocytes at the time of insemination and sex ratio of subsequent early bovine embryos. *Theriogenology* 47: 1041-1050.

Fulka, J. Jr., **First, N.L.**, C. Lee, J. Fulka, Moor, R.M. 1997. Induction of DNA replication in germinal vesicles and in nuclei formed in maturing mouse oocytes by 6-DMAP treatment. *Zygote* 5:213-217.

Fulka, J. Jr., Kalab, P., **First, N.L.**, Moor, R.M. 1997. Damaged chromatin does not prevent the exit from metaphase I in fused mouse oocytes. *Human Reproduction* 12:[11] 2473-2476.

Fulka, J. Jr., **First, N.L.**, Moor, R.M. 1996. Nuclear transplantation in mammals: remodeling of transplanted nuclei under the influence of maturation promoting factor. *BioEssays* 18:10.

Navara, C.S, **First, N.L.** and Schatten, G. 1996. Phenotypic variations among paternal centrosomes expressed within the zygote as disparate microtubule lengths and sperm aster organization: Correlations between centrosome activity and developmental success. *Proc. Natl. Acad. Sci. USA*. 93: 5384-5388.

Kent-First, M.J., Kol, S., Muallem, A., Ofir, R., Manor, D., Blazor, S., **First, N.**, Itsikovitz-Eldor, J. 1996. The incidence and possible relevance of Y-linked microdeletions in babies born after intracytoplasmic sperm injection and their infertile fathers. *Mol.Hum.Reprod.* 2(12):943-950.

Aktas, H., Wheeler, M.B., **First, N.L.**, Leibfried-Rutledge, M.L. 1995 Maintenance of Meiotic by increasing [cAMP]_i may have physiological relevance in bovine oocytes. *J.Reprod.Fert.* Schoff, P.K. and **N.L. First**. 1995. Manipulation of bovine sperm metabolism and motility using anoxia and phosphodiesterase inhibitors. *Cell Motility and the Cytoskeleton* 31:140-146.

Jones, J.M. and **N.L. First**. 1995. Expression of cell cycle control protein cdc25 in cleavage stage bovine embryos. *Zygote*. 3: May, 1995.

Hageman, L.J., Hillery, F.L., Leibfried-Rutledge, M.L. and **First, N.L.** 1994. Activation of murine oocytes with Ca2+ ionophore and cycloheximide. *J.Exper.Zool.* 271:57-61.

Susko-Parrish, J., Leibfried-Rutledge, M.L., Northey, D., Schutzkus, V. and **First, N.L.** 1994. Inhibition of protein kinases after an induced calcium transient causes transition of bovine oocytes to embryonic cycles without meiotic completion. *Developmental Biology*. 166: 729-739.

Rosenkrans, C.F. and **First, N.L.** 1994. Effect of free amino acids and vitamins on cleavage and developmental rate of bovine zygotes in vitro. *J. Anim. Sci.* 72: 434-437.

First, N.L., Sims, M.M., Park, S.P., and Kent-First, M.J. 1994. Systems for production of calves from cultured bovine embryonic cells. *Reprod. Fertil. and Dev.* 6: 553-562.

Sims, M.M. and **First, N.L.** 1994. Production of calves by transfer of nuclei from cultured inner cell mass cells. *Proc. Natl. Acad. Sci.* 90: 6143-6147.

Navara, C.S., **First, N.L.** and G. Schatten. 1994. Microtubule organization in the cow during fertilization, polyspermy, parthenogenesis and nuclear transfer: The role of the sperm aster. *Dev. Biol.* 162:29-40.

Sims, M.M., and **First, N.L.** 1994. Production of calves by transfer of nuclei from cultured inner cell mass cells. *Proc. Natl. Acad. Sci.* 90:6143-6147.

First, N.L., and Leibfried-Rutledge M.L. 1993. Nuclear Transfer in Mammals. In: "In vitro fertilization and Embryo Transfer in Primates" (D.P. Wolf, Stouffer R.L., and Brenner, R.M., Eds.), Springer-Verlag New York, Inc. 317-330.

Fulka Jr., J., Leibfried-Rutledge M.L., and **First, N.L.** 1993. Control of germinal vesicle breakdown in bovine x murine hybrid oocytes. *Reprod. Nutr. Dev.* 33:411-417.

Kim, T., M.L. Leibfried-Rutledge and **First, N.L.** 1993. Gene transfer in bovine oocytes using replication-defective retroviral vectors packaged with Gibbon ape leukemia virus envelopes. *Molec. Reprod. Dev.* 35:105-113.

Kim, T., M.L. Leibfried-Rutledge and **First, N.L.** 1993. Gene transfer in bovine oocytes using replication-defective retroviral vectors packaged with Gibbon ape leukemia virus envelopes. *Molec. Reprod. Dev.* 35:105-113.

Parrish, J.J. and First, N.L. 1993. Fertilization. Reproduction in domesticated animals. G. King ed. Elsevier Press, New York, pp. 195-228.

Prather, R.S. and **First, N.L.** 1993. Cell-to-cell coupling in early-stage bovine embryo: a preliminary report. *Theriogenology* 39:561-567.

Takahashi, Y. and **First, N.L.** 1993. In vitro fertilization of bovine oocytes in the presence of theophylline. *Anim. Reprod. Sci.* 34:1-8.

Takahashi, Y. and **First, N.L.** 1993. In vitro culture of bovine one-cell embryos fertilized in vitro using synthetic oviduct fluid medium supplemented with fetal calf serum. *Anim. Reprod. Sci.* 31:33-47.

Rosenkrans, Jr., C.F., G.Q. Zeng, G.T. McNamara, P.K. Schoff and **First, N.L.** 1993. Development of bovine embryos in vitro as affected by energy substrates. *Biol. Reprod.* 49:459-462.

Rosenkrans, Jr., CF., and **First, N.L.** 1993. Effect of free amino acids and vitamins on cleavage and developmental rate of bovine zygotes in vitro. *J. Anim. Sci.* 72:434-437.

Yom, H.C., R.D. Bremel and **First, N.L.** 1993. Mouse mammary tumor virus promoter directs high-level expression of bovine μ S1 casein in the milk of transgenic heterozygous and homozygous mice. *Anim. Biotech.* 4(1):89-107.

Hagemann, L.J. and **First, N.L.** 1992. Embryonic cytoplasmic extracts rescue murine androgenones to the blastocyst stage. *Development* 114(4): 997-1001

Takahashi, Y. and **First, N.L.** 1993. In vitro development of bovine one-cell embryos: influence of glucose, lactate, pyruvate, amino acids and vitamins. *Theriogenology* 39:561-567.

Eyestone, W.H., J.M. Jones and **First, N.L.** 1991. Oviduct-conditioned medium for the culture of early bovine embryos to the blastocyst stage. *J. Reprod. Fertil.* 92:59-64.

Eyestone, W.H. and **First, N.L.** 1991. Characterization of developmental arrest in early bovine embryos cultured in vitro. *Theriogenology* 35:613-624.

Hagen, D.R., R.S. Prather, M.M. Sims and **First, N.L.** 1991. Development of one-cell porcine embryos to the blastocyst stage in simple media. *J. Anim. Sci.* 69:1147-1150.

First, N.L. and R.S. Prather. 1991. Genomic potential in mammals. *Differentiation* 48:1-8.

Prather, R.S., M.M. Sims and **First, N.L.** 1991. Culture of porcine embryos from one and two cells to blastocysts in sheep oviducts. *Theriogenology* 35:1147-1152.

Fulka, J., Jr., M.L. Leibfried-Rutledge and **First, N.L.** 1991. Effect of 6-dimethylaminopurine upon germinal vesicle breakdown of bovine oocytes. *Mol. Reprod. Dev.* 29:379-384.

Barnes, F.L. and **First, N.L.** 1991. Embryonic transcription in in vitro cultured bovine embryos. *Molec. Reprod. Dev.* 29:117-123.

Hagen, D.R., R.S. Prather and **First, N.L.** 1991. Response of porcine oocytes to electrical and chemical activation during maturation in vitro. *Mol. Reprod. Dev.* 28:70-73.

Saeki, K., M. Hoshi, M.L. Leibfried-Rutledge and **First, N.L.** 1991. In vitro fertilization and development of bovine oocytes matured in serum-free medium. *Biol. Reprod.* 44:256-260.

Patents

Co-culture of embryos with other cell types
CR1aa embryos culture system
Nuclear transfer process
Activation of oocytes